IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Joanne Hama
Art Unit	:	1632
Applicants	:	William G. Kerr, John M. Ninos
Serial No.	:	10/605,452
Filed	:	September 30, 2003
For	:	Novel SH2containing Inositol 5'-phosphatase Isoform That Partners With The Grb2 Adapter Protein

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF WILLIAM G. KERR, Ph.D., UNDER 37 C.F.R. §1.132

Sir:

I, William G. Kerr, Ph.D., of University of South Florida, hereby declare:

THAT, I am a named inventor on the above-referenced patent application (hereinafter referred to as "the patent application");

THAT, through my years of research, I have kept up to date on the technical literature and maintained contact with experts in the field by participating in professional meetings and seminars, and by direct personal contact. As a result, I am familiar with the general level of skill of those working in the fields of immunology, stem cell biology, and cell signaling;

THAT, I have read and understood the specification and claims of the patent application and the Office Action dated April 12, 2006;

AND, being thus duly qualified, do further declare:

1. Claims 29, 32, 35, and 39-51 are rejected under 35 U.S.C. §112, first paragraph, as non-enabled. At page 4, the Office Action essentially indicates that the gene knockout data in the manuscript entitled "SHIP-deficiency enhances HSC Proliferation and Survival but Comprises Repopulating

Potential" (submitted as Exhibit B with the previous response), which was obtained using a Cre-lox mutation strategy, does not support or correlate with the use of RNAi for s-SHIP/SIP-110 gene silencing, as taught in the patent application. Specifically, the Office Action indicates that an artisan cannot predict: (1) that treating hematopoietic stem cells (HSC) with shRNA targeting s-SHIP/SIP-110 mRNA would have resulted in HSC proliferation; and (2) the level of RNAi-mediated gene silencing that is required such that a phenotype of expansion would occur. I respectfully disagree.

2. To appreciate the lines of evidence supporting the role of s-SHIP/SIP-110 in the growth and proliferation of embryonic stem cells (ESC) and HSC, one must consider the role of the full-length hematopoietic isoform of the SH2-containing inositol 5'phosphatase (SHIP) as a key signaling component and regulator of cellular responses in the mature cells of several hematopoietic lineages. As I indicated in the previous Declaration dated January 9, 2006, it has been known for 7-8 years that SHIP opposes phosphatidylinositol 3-kinase (PI3K) and thus, PI3K-effector pathways, which control cell proliferation and/or survival. Engagement of receptors on the surface of mammalian cells results in the activation of PI3K and phosphorylation of phosphatidylinositols on the cytoplasmic side of the cell membrane. The generation of phosphatidylinositol 3,4,5-triphosphate (PIP3) by PI3K contributes to the activation of signaling pathways that drive cell proliferation. SHIP can associate with various adapter proteins, scaffold proteins, or receptors following activation of hematopoietic cells. As described in the Tu *et al.* publication (*Blood*, 2001, 99(7):2028-2038, previously submitted), of which I am a co-author, and pages 3-4 of the aforementioned manuscript, formation of these complexes enables SHIP to hydrolyze the 5'-phosphate on PIP3, thus preventing membrane recruitment and activation of pleckstrin homology (PH) domain containing kinases that serve as effectors of PI3K signaling. SIP-110 was also shown to have enzymatic activity by Jefferson *et al.* (*J. Biol. Chem.*, 1997, 272(9):5983-5988, submitted herewith). This activity permits SHIP and SIP-110 to limit the survival, activation, differentiation, and/or proliferation of hematopoietic cells. Our laboratory and others have found that SHIP deficiency results in expansion of the HSC compartment *in vivo*. Furthermore, the results described in the manuscript of Exhibit B show that

SHIP-deficiency in the HSC of mice enhances HSC proliferation and survival. The published version of the manuscript (Desponts *et al.*, *Blood*, 2006, 107(11):4338-4345) is submitted herewith.

3. The data in the Tu *et al.* (2001) publication and pages 22-26 of the patent application make clear that the s-SHIP isoform is expressed from an internal site within the SHIP gene in embryonic stem cells (ESC) and HSC, but not in mature hematopoietic cells. The s-SHIP isoform lacks the SH2 domain found in the SHIP isoform whose expression is restricted to the hematopoietic system. Consistent with this structural difference, s-SHIP does not associate *in vivo* with the Shc adapter protein. However, like SHIP, s-SHIP does associate with the major adapter protein Grb2 and gp130 subunit of the LIF and c-kit receptors, but does not require tyrosine phosphorylation to do so (page 29, lines 7-15 of the patent application).

4. Kavanaugh *et al.* (*Curr. Biol.*, 1996, 6(4):438-445, submitted herewith) cloned and characterized the human SHIP isoform, SIP-110, that lacks an SH2 domain (GenBank accession number U50040). Kavanaugh *et al.* (1996) and Jefferson *et al.* (1997) also confirmed that SIP-110 binds with Grb2 and hydrolyzes PIP3, likely preventing PIP3 accumulation to significant levels (page 443, first column of the Kavanaugh *et al.* publication). Thus, like SHIP, SIP-110 opposes PI3K and, thus, PI3K-effector pathways, which control cell proliferation and/or survival. My laboratory's analysis of the structure and the genomic location of the first exon of SIP-110, which is provided in Figure 7 and paragraph [0070] at pages 33-34 of the patent application, confirmed that SIP-110 is the human homologue of s-SHIP. In view of the characterizing data obtained for SIP-110 and s-SHIP in the patent application and the scientific literature at the time the patent application was filed, it is reasonable to extrapolate between these two homologues. Furthermore, based on the data available for SIP-110 and s-SHIP in the patent application and the scientific literature at the time the patent application was filed, it is reasonable to conclude that s-SHIP/SIP-110 are utilized by ESC and HSC to oppose PI3K, thereby regulating cell proliferation and/or survival.

5. As described in the aforementioned manuscript, our laboratory has demonstrated that $SHIP^{-/-}$ mice generated using the Cre-lox method have an expanded HSC compartment. The Cre-lox method involves introducing loxP target sequences into the gene to be deleted, and engineering expression of the Cre recombinase enzyme under the control of a tissue-specific promoter. Thus, the enzyme is expressed only in the desired tissue, and it deletes the gene of interest via the loxP target sites. As indicated at page 16 of the manuscript, $SHIP^{-/-}$ mice were generated by deletion of the promoter and first exon of SHIP.

6. In contrast, RNAi affects the abundance of RNA and in turn, the abundance of protein. RNAi can be used to create "hypomorphs" (having reduced or weakened gene function) as well as "complete silencing". Potency and specificity of gene silencing are the major advantages of the RNAi methodology over other nucleic acid-based gene targeting approaches. This is evidenced by the following scientific reports and review papers, which are submitted herewith: Diallo *et al.*, "Long endogenous dsRNAs can induce complete gene silencing in mammalian cells and primary cultures", *Oligonucleotides*, 2003, 13(5):381-392; Scherr *et al.*, "Inhibition of GM-CSF receptor function by stable RNA interference in a NOD/SCID mouse hematopoietic stem cell transplantation model", *Oligonucleotides*, 2003, 13(5):353-363; Bot *et al.*, "Lentiviral shRNA silencing of murine bone marrow cell CCR2 leads to persistent knockdown of CCR2 function *in vivo*", *Blood*, 2005, 106(4):1147-1153; Soutschek *et al.*, "Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs", *Nature*, 432:173-178; McCaffrey and Kay, "RNA interference gets infectious", *Gene Therapy*, 10:1205; Kim, "RNA interference in functional genomics and medicine", *J. Korean Med. Sci.*, June 2003, 18:309-318; Sandy *et al.*, "Mammalian RNAi: a practical Guide", *BioTechniques*, 2005, 39:215-224; Lenz, "The RNA interference revolution", *Brazilian Journal of Medical and Biological Research*, 205, 38:1749-1757; and Zhou *et al.*, "RNAi technology and its use in studying the function of nuclear receptors and coregulators", *Nuclear Receptor Signaling Atlas*, September 2003, 1:e008.

7. Given the state of the art as demonstrated by the scientific publications submitted herewith and previously, and the information provided in the patent application and the experimental results obtained therewith, one of ordinary skill in the art can target and reduce expression of s-SHIP/SIP-110 without resort to undue experimentation. Furthermore, based on the experimental results in Figure 8 of the patent application, and the state of the art of RNAi technology at the time the patent application was filed, one of ordinary skill in the art would reasonably expect that sufficient s-SHIP/SIP-110 knockdown could be achieved to induce proliferation, growth, and/or survival of ESC and HSC.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or of any patent issuing thereon.

Further declarant sayeth naught.

Signed: William D Kerr

William G. Kerr, Ph.D.

Date: 7/10/06